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Alleviation The Adverse Effects Of Salinity Stress In Sunflower Cultivars Using Nicotinamide And α-Tocopherol

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Abstract: A possible survival strategy of plants under saline conditions is to use some compounds that could alleviate salt stress effect. The effect of exogenously application of α-tochopherol or nicotinamide as seed soaking prior to sowing in saline soil with different salinity levels (EC 1.56, 4.68 and 7.83 ds/m) to sunflower (*Helianthus annuus* L.) cultivars (Hysun 336 and Euroflor) was investigated. Salinity stress decreased total soluble sugars content concomitantly with increasing proline, free amino acids and total phenol contents in both cultivars. The activities of CAT, POX, PPO and PAL were decreased with increasing salinity level. In addition, the content of Na^+ was increased significantly under salinity stress, while, N, P, K and Mg contents were decreased with increasing salinity levels. Also, salinity stress decreased the contents of some microelements (Fe, Mn, Zn and Cu) at both cultivars. Soaking sunflower seeds with different concentrations of α-tocopherol or nicotinamide could alleviate the harmful effects of salinity stress.

Key words: Sunflower, Salinity, Vitamins, Antioxidants.

INTRODUCTION

High salt concentrations in soils inhibiting crop growth and productivity are frequent constraint to agriculture in arid and semi-arid regions. Irrigation with poor quality water is one of the main factors resulting in salt accumulation and decrease of agricultural productivity. As saline soils and saline waters are common around the world, great efforts have been devoted to understand physiological aspects of tolerance to salinity in plants. Salinity imposes both ionic and osmotic stresses on plants (Munns *et al.,* 2006). Therefore, salinity affects almost every aspect of the physiology and biochemistry of plants. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generates and transmits signals and instigates biochemical changes that adjust the metabolism accordingly (Dolatabadian & Saleh 2009). Reactive oxygen species such as superoxide radical (O^-_2), hydrogen peroxide (H_2O_2) and hydoxyl (OH^-) radical are also produced during salinity stress, and are responsible for the damage to membranes and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids. ROS scavenge by various antioxidant enzymes such as CAT, POX and PPO. Changes in activities of various antioxidant enzymes under salinity stress have been reported earlier (Dolatabadian & Saleh 2009).

Sunflower is a major oil seed crop worldwide, and it is also an important crop in Mediterranean areas where salinity is an increasing problem (Caterina *et al*., 2007). Sunflower is moderately sensitive to soil salinity, where it can tolerate salinity up to EC equal to 1.7 dsm^{-1}. The promotion of sunflower could be successful to increase the domestic production provided proper cultivars are available which are suitable to different soil and climatic conditions (Khatoon *et al*., 2006).

There is strong evidence that in many crop plants natural accumulation of osmoprotectants and other organic compounds is very low and this deficiency can be overcome by their exogenous application. Exogenous applications of vitamins have been reported to successfully mitigate the adverse effects of salinity on plants (Khan *et al*., 2006). Vitamins are organic compounds which are required in trace amount to maintain normal growth and proper development of all organisms; these compounds act as coenzyme systems and thus take essential part in the regulation of metabolism, vitamins, can be limiting factors in the development of plant (Hassanein *et al*., 2009). Presoaking of seeds with optimal concentration of vitamins has been shown to be beneficial in seedling growth under saline condition by increasing physiological availability of water and nutrient. From the earlier mentioned reports it is evident that nicotinamide (Vit. pp) counteracted the adverse effects of salinity on seed germination, seedling growth and some relevant metabolic activities (Bassouny *et al*., 2008). α-Tocopherol (vitamin E) is an amphiphilic lipid antioxidant. Increasing evidence suggests that in higher plants vitamin E might play a protective role to membrane system in cell. This evidence points at α-tocopherol being an important part of the plant defense machinery maintaining the integrity and normal function of the photosynthetic apparatus (Collin *et al*., 2007).

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Thus, the main objective of the present study was to examine whether the adverse effects of salt stress on two sunflower cultivars could be mitigated by exogenous application of nicotinamide and α-tocopherol as a seed soaking and how far it regulates the plant antioxidant enzyme system, some metabolites, microelement and macroelement contents.

MATERIALS AND METHODS

Experimental Conditions:

Two field experiments were conducted at the Agricultural station of Agricultural Faculty, Fayoum University, Fayoum Governorate, Egypt. During two successive seasons, three experimental sites were chosen with physical and chemical analysis as shown in (Table 1). Soil analysis was carried out according to (Black *et al*., 1965 and Jackson, 1973).

Table 1: Mechanical And Chemical Analysis Of Soils.

Properties	Site 1	Site 2	Site 3
Mechanical Analysis			
Coarse Sand%,	3.15	3.75	2.85
Fine	63.85	65.25	47.15
Silt%	19.75	20.25	20.50
Clay%	13.25	10.75	29.50
Soil texture	Sand loamy	Sand loamy	Sand clay loamy
pH (1:2.5)	7.36	7.64	7.81
EC (ds/m)	1.56	4.68	7.83
Chemical Analysis			
Organic matter%	1.42	1.38	1.25
$CaCO_3$	9.34	8.56	8.05
Total N%	0.09	0.07	0.06
P	5.16	7.02	8.36
K	201.24	198.1	181.15
Fe	7.03	5.94	5.37
Mn	1.52	1.04	0.98
Zn	0.88	0.79	0.76
Cu	0.67	0.59	0.63

At soil preparation fertilizers supplemented with full dose 200 kg/fed of calcium superphosphate (15.5% P_2O_5), 200 kg/fed ammonium nitrate (33.5% N) and 50 Kg/fed potassium sulphate (48% K_2O) were incorporated into the top 15 cm of the soil. Normal agricultural practices common in the area were followed.

Seeds of the two cultivars of sunflower (Hysun 336 and Euroflor) were carried from Agricultural Research Centre Assuit branch, Egypt. Chemical compounds (α-tocopherol and nicotinamide) were supplied from SIGMA – ALDRICH Company. Soaking of seeds of the two cultivars were for 12 h in different concentrations of α-tocopherol (0.0, 25 and 50 mg/l) or nicotinamide (0.0, 2.5 and 5 mg/l). Seeds of the two cultivars were separately sown in the mentioned three experimental sites at two successive seasons. The seeds of the two cultivars were sown in split split plot design with four replications in rows 4-meter long, 0.60-meter apart and 6 ridges with total area (14.4 m^2). Hill spacing was 10 cm within the row. Seeds were sown at 3-5 seeds in each hill. The sites of each experiment put as main plot, vitamins as subplot and concentrations of α-tocopherol or nicotinamide as sub sub plot. Irrigation took place immediately after sowing, then every one week's intervals according to agronomic practices in the district. Thinning was carried out at 15 days after sowing to secure two plants per hill on both sides of the ridge.

Plant Sampling:

Four plant samples/plot were harvested 50 days after sowing for chemical analysis. Determination of total soluble sugars, total amino acids, total protein, macro (N, P, K, Na, Mg and Ca) and microelements (Fe, Mn, Zn and Cu) in the dry tissues were determined. Total phenols & proline contents and some enzyme activities (CAT, POX, PPO and PAL) also determined in the fresh tissues.

Chemical Analysis:

Total soluble sugars (TSS):

Total soluble carbohydrates (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme *et al*. 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and

reading the cooled samples at 625 nm using Spekol SpectrocolorimeterVEB Carl Zeiss (Yemm and Willis,1994).

***Total Phenol*:**

A known weight of the fresh samples of shoots were taken and extracted with 85% cold methanol (v/v) for three times at 0°C. The combined extracts were collected, dried under vacuum and made up to a known volume with distilled water. Then 0.5 ml of the extraction was added to 0.5 ml Folin, shaken allowed to stand for 3 min. Then one ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60min. The optical density was determined at wave length of 725 nm using spectrophotometer as described by Danil and George (1972).

Proline:

Proline was assayed according to the method described by Bates *et al*. (1973). 2ml of proline extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocolourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Free Amino Acids:

Free amino acid content was extracted according to the method described by Vartanain *et al*. (1992). Free amino acid was determined with the ninhydrin reagent method (Yemm & Cocking 1955). 1 ml acetate buffer (pH 5.4) and 1 ml chromogenic agent were added to 1 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. after cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocolourimeter VEB Carl Zeiss.

Total Protein:

Total protein concentration of the supernatant was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard. An amount of 2 gm of samples were grinded in mortar with 5ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant of different samples were put in separate tubes. The volume of all of the samples in tubes were then made equal by adding phosphate buffer solution and the extraction were stored in the refrigerator at 40c for further analysis. After extraction, 30µl of different samples were taken out in separate tubes and were mixed with 70µl of distilled water separately. In all of these separate sample tubes 2.9 ml of Coosmassic Brillaint Blue solution was then added and mixed thoroughly. The Total volume now was 3ml in each tube. All these tubes were incubated for 5 minutes at room temperature and absorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus Concentration (µg) of protein was calculated.

Assay Of Enzymes Activities:

Enzyme extractions were collected following the method described by Chen and Wang (2006). Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8,000 rpm and 4°C for 15 min. The supernatant was used immediately to determine the activities of enzymes.

Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva *et al*., 2001). Phosphate buffer solution pH 7 (0.1 M, 1.95 ml), 1 ml of 0.1 M pyrogallol as a substrate and 50 µl of the enzyme extract were pipetted into a test tube and mixed thoroughly. Then the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 410 nm was recorded continuously at 25°C for 5 min.

Peroxidase (POX, EC 1.11.1.7) activity was assayed by the method of Kumar and Khan (1982). The reaction mixture used for estimating the peroxidase enzyme (POX) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25°C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a reagent blank prepared by adding the extract after the addition of 2.5 N H2SO4 at the zero time.

Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm (Chen and Wang 2006). The mixture (3 ml) contained 1.9 ml phosphate buffer (50 mM, pH7.0), 100 µl enzyme extract, and 1 ml 0.3% H_2O_2. The reaction was initiated by adding enzyme extract. One unit of CAT activity was defined as the 0.01 deduction in absorbance at 240 nm per minute.

Phenylalanine ammonialyase **(**PAL, EC 4.3.1.5) activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290nm (Dickerson *et al*., 1984). Sample containing 0.4ml of enzyme extract was incubated with 0.5ml of 0.1M borate buffer, pH-8.8 and 0.5ml of 12mM L-phenyl alanine in the same buffer for 30 min at 300C. The amount of trans-cinnamic acid synthesized was calculated using its

extinction coefficient of 9630 M^{-1} cm^{-1}. Enzyme activity was expressed as synthesis of trans-cinnamic acid (in nmol quantities) min^{-1} g^{-1} fresh weight. The enzyme activities were calculated by Kong *et al* (1999).

Mineral Ions:

Macro and microelement contents of were determined according to the method described by Chapmen & Pratt (1978). N and P were determined using Spekol Spectrocolourimeter VEB Carl Zeiss. While, estimation of Ca, K and Na contents were done by the use of flame photometer. Also, Mg, Fe, Mn, Zn and Cu contents were estimated using atomic absorption spectrophotometer.

Statistical Analysis:

The data were statistically analyzed on split split plot design according to Snedecor & Cochran (1980). Means were compared by least significant difference (LSD) at 5% levels of probability (Duncan 1955).

Results:

Total Soluble Sugars:

Data Fig.1A show the response of sunflower plant to salinity stress and the effect of α-tocopherol and nicotinamide on total soluble sugar contents. A difference in total soluble sugars content between the two sunflower cultivars was observed. Increased salinity levels from EC 1.56 to 4.68 to 7.83 ds/m significantly reduced total soluble sugars contents of Hysun 336 cultivar as well as of Euroflor cultivar. The percentages of decrease were 18.24% & 25.68%in Hysun and by 14.08% & 23.39% in Euroflor cultivar under salinity levels 4.68 & 7.83 ds/m, respectively.

The data herein obtained revealed that pretreatment of both sunflower cultivar seeds with the different concentrations of α-tocopherol (25 and 50 mg/l) and nicotinamide (2.5 and 5.0 mg/l) stimulated the accumulation of total soluble sugars as compared with the corresponding salinity level. Meanwhile, more total soluble sugars accumulated in cultivar Hysun 336 as compared with Euroflor cultivar in all salinized and treated sunflower plants. It is worthy to mention here that, α – tocopherol (50 mg/l) induced the most pronounced effect under all salinity levels at two cultivars Fig. 1 A.

Total Phenol:

In the present study, total phenol content of sunflower cultivars was significantly increased gradually with the increase of salinity level. The percent of increase reached to 15% and 28% in Hysun 336 cultivar and to 15% and 30% in Euroflor cultivar when rising EC of soil from 1.56 to 4.68 and 7.83 ds/m respectively as shown by Fig.1 B. Priming seeds of two cultivars by soaking it in α-tocopherol (25 & 50 mg/l) or nicotinamide (2.5 & 5.0 mg/l) has no effect on total phenol content of two cultivars as compared with the corresponding salinity levels Fig. 1B.

Proline, Total Amino Acids And Total Protein:

Data recorded in the present study Figs. 2 A & B. indicated that salt stress induced accumulated amounts of both proline and free amino acid in plant with increasing salt stress. Application of α-tocopherol or nicotinamide as seed soaking induced an additive stimulatory effect on the accumulation of proline and free amino acids contents as compared with those of the corresponding salinity level.

Increasing soil salinity led to gradual significant decrease in the total protein content in both sunflower cultivars Fig. 2C. Pretreatment of both sunflower seed cultivars with either Vit E (50 mg/l) or Vit.PP (2.5 and 5.0 mg/l) induced significant increases in total protein content of sunflower plant under all salinity levels as compared with the corresponding salinity level.

Enzyme Activities:

The changes in the activities of the various enzymes in response to salinity stress either alone or in combination with each of the two vitamins α-tocopherol and nicotinamide are illustrated Fig. 3. Results indicated that, catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO) and phenylalanine ammonialyase (PAL) were significantly decreased under stress conditions in both cultivars. Priming of sunflower seeds with α-tocopherol or nicotinamide improve stress resistance by the increase in CAT, POX, PPO and PAL activities as compared with corresponding salinity level. The higher activities were recorded in response to 50 mg/l α-tocopherol and 5.0 mg/l nicotinamide in CAT, POX and PPO in both cultivars. At the same time, nicotinamide has more pronounced effect than α-tocopherol treatment on PAL activity.

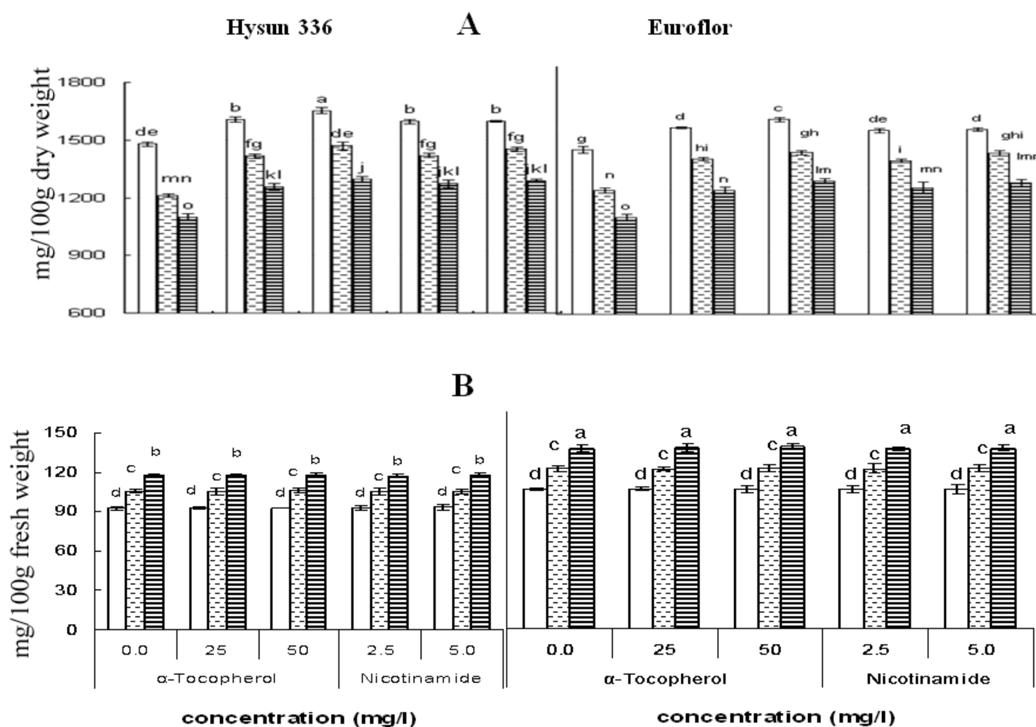

Fig. 1: Effect of α- Tocopherol and nicotinamide on chemical contents (A) total soluble sugar, (B) total phenol of two sunflower cultivars (Hysun 336 and Euroflor) under different levels of soil salinity (clear bar =1000 ppm ,dashed horizontal bar = 3000 ppm and dark horizontal bar = 5000 ppm) . Each value represents the mean ± standard error. Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Mineral Contents:

Salinity stress caused significant decreases in nitrogen, phosphorous, calcium and magnesium contents of two sunflower cultivars with the increases in soil salinity levels Table 2. Pretreatment of sunflower seeds with either α-tocopherol (50 mg/l) or nicotinamide (2.5 and 5.0 mg/l) induced significant accumulation of N under all salinity levels in both cultivars as compared with the corresponding salinity level. The same trend was observed in response to P with both concentrations of either Vit. E or Vit PP. Regarding the effect of soaking seeds of both sunflower cultivars on Ca and Mg contents the results recorded non significant variation between treatments as compared with the corresponding salinity level.

Results Table 3 showed the response of K^+, Na^+ and K^+/Na^+ ratio of both sunflower cultivars subjected to different salinity levels. The significant accumulation of sodium increased with increasing salinity level in both cultivars. This accumulation of Na^+ content was accompanied with significant gradual decrease in K^+ content and K^+/Na^+ ratio in both cultivars. In the meantime, Hysun 336 cultivar showed higher significant values of K^+/Na^+ ratio as compared with Euroflor cultivar. Soaking sunflower seeds in either Vit.E or Vit PP showed significant decrease in Na^+ content in both cultivars at EC 4.68 and 7.83 ds/m levels of salinity. Pretreatment of seeds with either α-tocopherol (25 and 50 mg/l) or nicotinamide (2.5 and 5.0 mg/l) induced significant increases in K^+ and K^+/Na^+ ratio in both cultivars as compared with the corresponding salinity level.

With regard to the effect of salinity on microelement contents of sunflower cultivars, data Table 4 revealed that, increasing salinity level caused gradual decrease in Fe, Mn, Zn and Cu contents in both cultivars. In the mean time, soaking both cultivars of sunflower seeds in either Vit E or Vit.PP caused slight increase in the above microelement as compared with the corresponding salinity level. This increase in Fe content was significant by using higher concentration of either α-tocopherol (50 mg/l) or nicotinamide (5.0 mg/l) under all salinity level. Seed soaking in higher concentration of either Vit. PP or Vit E induced significant increases in the Mn, Zn and Cu contents under 1.56 and 4.68 ds/m salinity level. While use the lower concentration of both vitamins under all salinity levels or high concentration of them under the highest salinity level (7.83 ds/m) induced non significant increase in the above mentioned microelement as compared with the corresponding salinity level.

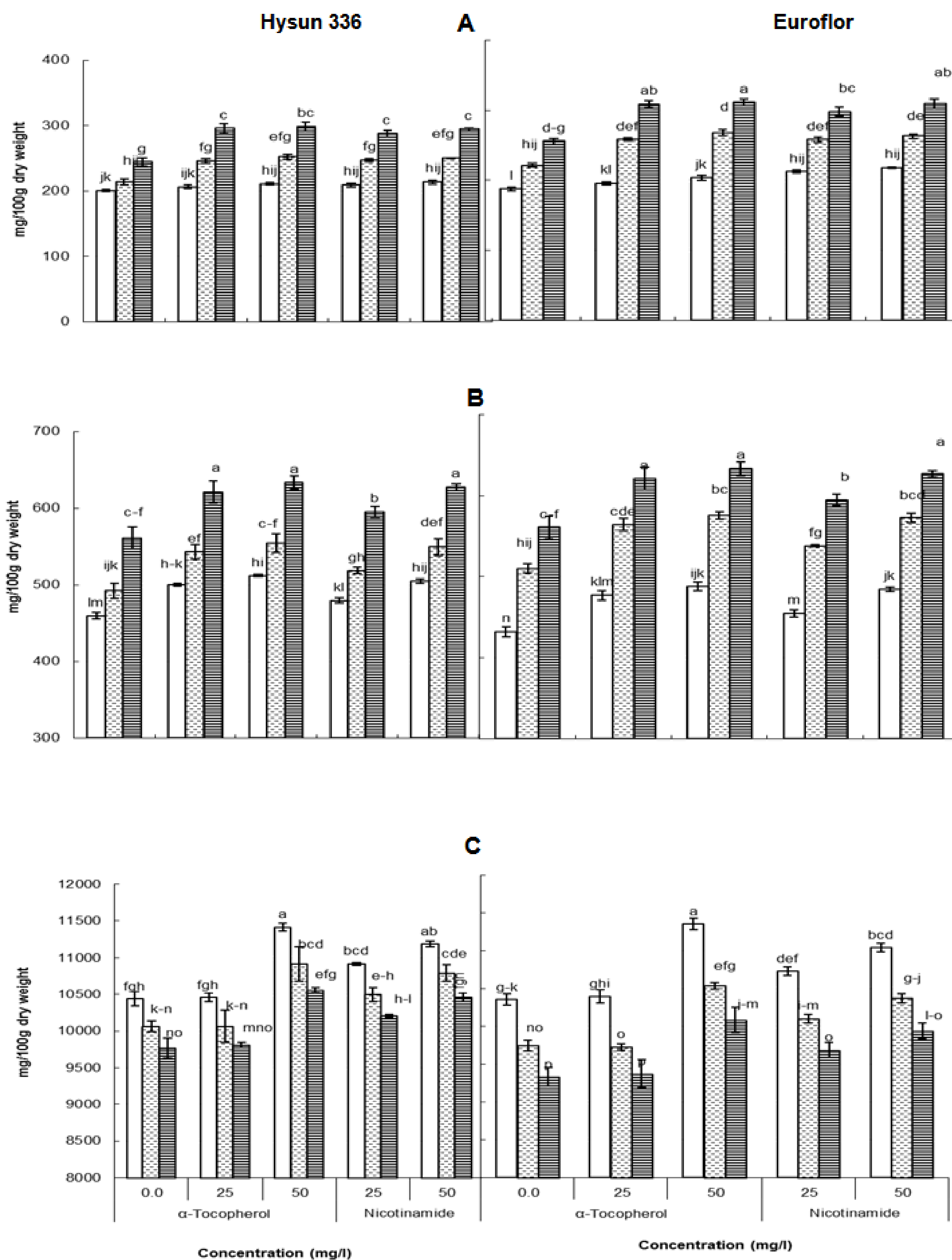

Fig. 2: Effect of α- Tocopherol and nicotinamide on chemical contents(A)proline , (B) total amino acid (C) total protein of two sunflower cultivars (Hysun 336 and Euroflor) under different levels of soil salinity (clear bar =1000 ppm ,dashed horizontal bar = 3000 ppm and dark horizontal bar = 5000 ppm) . Each value represents the mean ± standard error. Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

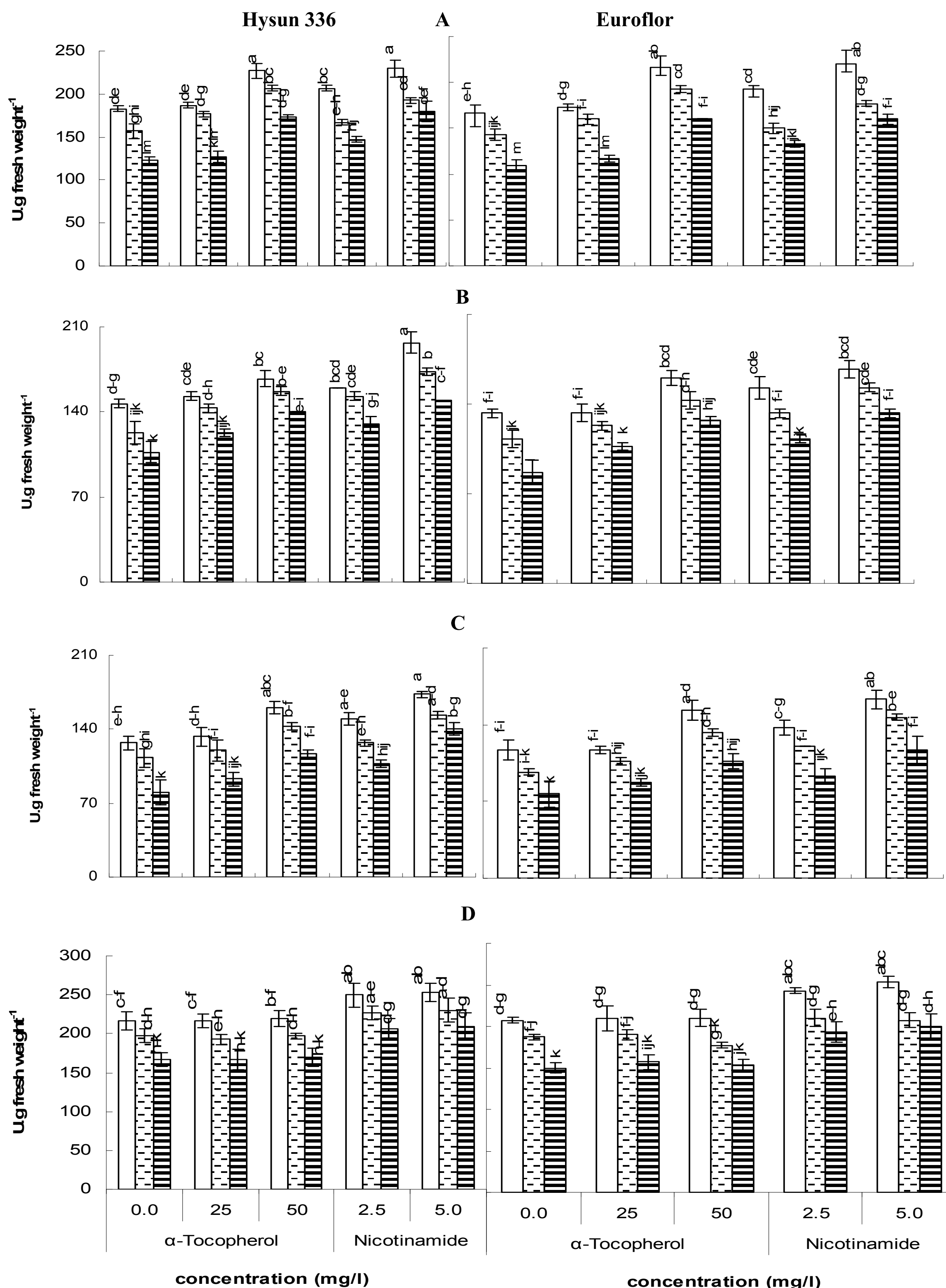

Fig. 3: Effect of α- Tocopherol and nicotinamide on enzyme activities (A) Catalase, (B) Peroxidase (C) Polyphenol Oxidase, (D) Phenyl alanine ammonia layase of sunflower cultivars (Hysun 336 and Euroflor) under different levels of soil salinity (clear bar =1000 ppm ,dashed horizontal bar = 3000 ppm and dark horizontal bar = 5000 ppm) . Each value represents the mean ± standard error. Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Table 2: Effect of α- tocopherol and nicotinamide on some macroelement contents of sunflower cultivars (Hysun 336 and Euroflor) under different levels of soil salinity. Each value represents the mean ± standard error. Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Treatment			N		P		Ca		Mg	
Salinity ppm	Material (mg/l)		mg/100g dry weight							
			Hysun 336	Euroflor	Hysun 336	Euroflor	Hysun 336	Euroflor	Hysun 336	Euroflor
1000	α-Tocopherol	0.0	1670 ± 15 fgh	1657 ± 12 g-k	1670 ± 88 f-i	1530 ± 33 i-l	2383 ± 19 a	2127 ± 26 b	325 ± 3 a	315 ± 3 abc
		25	1673 ± 9 fgh	1663 ± 15 ghi	2000 ± 58 abc	1800 ± 58 d-g	2383 ± 7 a	2130 ± 26 b	326 ± 3 a	316 ± 3 abc
		50	1827 ± 9 a	1817 ± 12 a	2130 ± 88 abc	1930 ± 33 bcd	2390 ± 45 a	2127 ± 9 b	326 ± 1 a	317 ± 4 ab
	Nicotinamide	2.5	1747 ± 3 bcd	1717 ± 9 def	1870 ± 88 cde	1670 ± 33 f-i	2390 ± 15 a	2133 ± 33 b	326 ± 3 a	316 ± 3 abc
		5.0	1790 ± 6 ab	1767 ± 9 bcd	2070 ± 67 abc	1870 ± 33 cde	2390 ± 15 a	2137 ± 24 b	327 ± 3 a	317 ± 4 ab
3000	α-Tocopherol	0.0	1610 ± 12 k-n	1560 ± 12 no	1570 ± 33 h-k	1400 ± 58 k-n	1897 ± 12 c	1763 ± 35 d	313 ± 2 ab	302 ± 3 bcd
		2.5	1610 ± 35 k-n	1557 ± 7 o	1830 ± 33 c-f	1630 ± 33 g-j	1900 ± 26 c	1767 ± 19 d	314 ± 4 ab	302 ± 1 bcd
		5.0	1747 ± 37 bcd	1687 ± 7 efg	1930 ± 67 bcd	1800 ± 58 d-g	1903 ± 22 c	1767 ± 18 d	315 ± 4 ab	303 ± 6 bcd
	Nicotinamide	25	1680 ± 15 e-h	1617 ± 9 i-m	1830 ± 33 c-f	1570 ± 33 h-k	1903 ± 38 c	1767 ± 9 d	314 ± 1 ab	302 ± 5 bcd
		50	1727 ± 18 cde	1660 ± 10 g-j	1930 ± 33 bcd	1730 ± 120 e-h	1903 ± 29 c	1803 ± 15 d	314 ± 3 ab	301 ± 3 cd
5000	α-Tocopherol	0.0	1563 ± 22 no	1493 ± 20 p	1270 ± 67 mn	1230 ± 67 n	1633 ± 20 e	1537 ± 32 f	292 ± 4 d	270 ± 7 e
		25	1570 ± 6 mno	1500 ± 29 p	1500 ± 100 i-l	1370 ± 67 lmn	1633 ± 9 e	1540 ± 21 f	292 ± 6 d	271 ± 6 e
		50	1690 ± 6 efg	1613 ± 26 j-m	1670 ± 120 f-i	1470 ± 33 jkl	1637 ± 15 e	1543 ± 27 f	292 ± 7 d	270 ± 8 e
	Nicotinamide	2.5	1633 ± 3 h-l	1550 ± 15 o	1430 ± 88 klm	1400 ± 58 k-n	1637 ± 7 e	1543 ± 37 f	292 ± 2 d	271 ± 6 e
		5.0	1673 ± 9 fgh	1590 ± 17 l-o	1530 ± 33 i-l	1470 ± 67 jkl	1643 ± 9 e	1543 ± 3 f	293 ± 8 d	271 ± 5 e

Discussion:

*Total Soluble Sugars***:**

In view of the results obtained from this study, it is obvious that salt stress caused a reduction in the total soluble sugars of both sunflower cultivars Fig.1A. The change in total soluble sugar contents under salt stress has already been reported for a number of plant species (Khattab, 2007 and Hassanein *et al*., 2009). This reduction concluded that salt stress may inhibit the photosynthetic activity and/or increased partial utilization of carbohydrates into other metabolic pathways (Hassenein *et al*., 2009). Marschner (1995) reported that the organic acids especially sugars are the main solutes involved in osmotic adjustment in some plants submitted to osmotic and saline stress. Exogenous application of vitamins is considered to be an alternative short-term solution to induce salt tolerance in some of the important crop cultivars (Waseem *et al*., 2006). Vitamin E and vitamin PP application generally stimulated the accumulation of total soluble sugars in the salt-affected sunflower cultivars, either via increasing endogenous levels of certain phytohormones or by acting as activators of carbohydrates synthesis (Hassenein *et al*., 2009). Moreover, accumulation of carbohydrate play a key role in alleviating the salinity stress, either via osmotic adjustment (Ackerson, 1985) or by conferring some desiccation resistance to plant cells (Srivastava *et al*.,1995).

Total Phenols:

Total phenols play a significant role in the regulation of plant metabolic processes and overall plant growth (Lewis & Yamamoto, 1990) Fig.1B. Our obtained data show significant increases in total phenol contents with the increase in salinity levels. These obtained data are in good agreement with those obtained by Mohamed & Aly (2008) on onion plant and El Hariri *et al*. (2010) on flax plant. Phenols act as a substrate for many antioxidants enzymes, so, it mitigates the salinity stress injuries (Lewis & Yamamoto, 1990). In this connection

phenol protect cells from potential oxidative damage and increase stability of cell membrane (Burguieres *et al*., 2006). Moreover, Rivero *et al*. (2001) recorded that an accumulation of phenolic compounds in response to abiotic stress. This would be beneficial to achieve acclimatization and tolerance to salt stress, since many kinds of plant phenolic have been considered to be the main lines of cell acclimatization against stress in plant.

Table 3: Effect of α-tocopherol and nicotinamide on potassium and sodium contents and K/Na ratio of sunflower cultivars (Hysun 336 and Euroflor) under different levels of soil salinity. Each value represents the mean ± standard error. Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Treatment			K^+		Na^+		K^+/Na^+	
			mg/100g dry weight					
Salinity ppm	Material (mg/l)		Hysun 336	Euroflor	Hysun 336	Euroflor	Hysun 336	Euroflor
1000	α-Tocopherol	0.0	1200 ± 12 [e-h]	1153 ± 9 [g-j]	233 ± 4 [p-s]	252 ± 5 [p]	5.15 ± 0.08 [c]	4.59 ± 0.09 [e]
		25	1343 ± 12 [a]	1250 ± 36 [b-e]	232 ± 2 [rs]	250 ± 6 [pqr]	5.80 ± 0.06 [a]	5.01 ± 0.26 [cd]
		50	1387 ± 9 [a]	1287 ± 26 [b-e]	232 ± 5 [qrs]	251 ± 5 [pr]	5.98 ± 0.14 [a]	5.14 ± 0.20 [cd]
	Nicotinamide	2.5	1277 ± 15 [bc]	1203 ± 23 [e-h]	231 ± 4 [s]	250 ± 7 [qr]	5.53 ± 0.04 [b]	4.82 ± 0.21 [de]
		5.0	1360 ± 10 [a]	1270 ± 17 [bc]	232 ± 6 [rs]	250 ± 6 [pqr]	5.88 ± 0.14 [a]	5.09 ± 0.16 [cd]
3000	α-Tocopherol	0.0	1127 ± 20 [ijk]	1073 ± 12 [kl]	436 ± 10 [i]	469 ± 7 [h]	2.58 ± 0.06 [h]	2.29 ± 0.05 [l]
		25	1257 ± 20 [b-e]	1197 ± 15 [e-h]	402 ± 5 [klm]	429 ± 4 [ij]	3.13 ± 0.05 [g]	2.79 ± 0.05 [h]
		50	1290 ± 26 [b]	1237 ± 15 [b-f]	387 ± 8 [m]	405 ± 4 [kl]	3.34 ± 0.08 [g]	3.06 ± 0.05 [g]
	Nicotinamide	2.5	1207 ± 3 [d-g]	1137 ± 7 [ij]	393 ± 6 [lm]	416 ± 3 [jk]	3.07 ± 0.04 [g]	2.73 ± 0.02 [h]
		5.0	1267 ± 9 [bcd]	1223 ± 18 [c-f]	334 ± 7 [o]	370 ± 5 [n]	3.79 ± 0.06 [f]	3.31 ± 0.05 [g]
5000	α-Tocopherol	0.0	1063 ± 15 [lm]	1013 ± 34 [m]	710 ± 4 [b]	727 ± 7 [a]	1.50 ± 0.01 [lm]	1.39 ± 0.05 [m]
		25	1183 ± 15 [f-i]	1120 ± 31 [jkl]	675 ± 5 [cd]	679 ± 9 [a]	1.75 ± 0.01 [jkl]	1.65 ± 0.07 [j-m]
		50	1223 ± 9 [c-f]	1143 ± 35 [hij]	647 ± 4 [ef]	654 ± 3 [ef]	1.89 ± 0.01 [jkl]	1.75 ± 0.05 [jkl]
	Nicotinamide	2.5	1127 ± 3 [ijk]	1067 ± 19 [klm]	660 ± 2 [de]	664 ± 7 [cde]	1.71 ± 0.00 [jkl]	1.61 ± 0.04 [klm]
		5.0	1207 ± 12 [d-g]	1133 ± 32 [ij]	628 ± 2 [g]	640 ± 6 [fg]	1.92 ± 0.02 [j]	1.77 ± 0.05 [jkl]

Application of α-tocopherol or nicotinamide under the various levels of salinity caused non significant changes in phenol contents in both sunflower cultivars as compared with those of the corresponding salinity levels. Therefore, the treatment with vitamins could alleviate the adverse effect of salinity on growth and metabolic activities through decreasing the building-up active oxygen species and thereby increasing resistance of salt stress (Hassanein *et al*., 2009).

Proline:

With regard to proline content, there is a strong correlation between increased cellular proline levels and the capacity to survive the effects of high environmental salinity. It may also, serve as nitrogen reserve (Sairam & Tyagi, 2004). In sunflower plant, proline accumulation was observed in all stressed plants of the two cultivars compared with low level of soil salinity Fig. 2A. Similar results have been reached by Khattab, (2007) and Sadak *et al*., (2010) on different plant species. The higher level of proline content in sunflower shoots may be due to expression of gene encoding key enzymes of proline synthesis and low activity of the oxidizing enzymes which is controlled by osmotic and salinity stress (Amini & Ehasapour (2005). Proline also can play a role as protective agent for cytoplasmic enzymes (Nikolopoulos and Manetase, 1991) and/or scavenging hydroxyl radicals (Hoque *et al*., 2007). Thus, it could be suggested that salt tolerance was manifested via activated proline synthesis and hydrolysis of protein into free amino acids to act as osmoprotectants in the different organs of the tested sunflower plant. This means that the inhibitory effect of salt stress on the tested sunflower plant was

alleviated by vitamins treatments through increasing proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein. Finally, proline able to activate multiple responses that are component of the adaptation processes (Maggio *et al*., 2002).

Table 4: Effect of α-Tocopherol and nicotinamide on microelement contents of sunflower cultivars (Hysun 336 and Euroflor) under different levels of soil salinity. Each value represents the mean ± standard error. Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Treatment			Fe		Mn		Zn		Cu	
			mg/100g dry weight							
Salinity ppm	Material (mg/l)		Hysun 336	Euroflor	Hysun 336	Euroflor	Hysun 336	Euroflor	Hysun 336	Euroflor
1000	α-Tocopherol	0.0	51.37 ± 4 ^{bc}	49.23 ± 3 ^{def}	36.93 ± 5 ^{b}	33.63 ± 7 $^{c-g}$	25.90 ± 4 ^{b}	23.90 ± 5 ^{cd}	18.27 ± 4 $^{c-f}$	17.43 ± 3 ^{ef}
		25	51.60 ± 3 ^{bc}	49.37 ± 5 ^{de}	37.03 ± 6 ^{b}	33.80 ± 7 ^{cde}	26.03 ± 5 ^{b}	23.97 ± 6 ^{c}	18.33 ± 4 $^{c-f}$	17.50 ± 3 ^{def}
		50	54.30 ± 3 ^{a}	51.97 ± 5 ^{b}	39.20 ± 6 ^{a}	35.73 ± 7 ^{bc}	27.83 ± 5 ^{a}	25.53 ± 6 ^{b}	19.53 ± 4 ^{ab}	18.60 ± 3 ^{bcd}
	Nicotinamide	2.5	51.80 ± 4 ^{b}	49.43 ± 8 ^{de}	37.07 ± 5 ^{b}	33.70 ± 10 $^{c-f}$	26.07 ± 7 ^{b}	23.90 ± 7 ^{cd}	19.43 ± 3 ^{ab}	18.47 ± 3 $^{b-e}$
		5.0	53.70 ± 5 ^{a}	51.23 ± 8 ^{bc}	40.73 ± 4 ^{a}	36.83 ± 7 ^{bc}	27.97 ± 7 ^{a}	25.70 ± 7 ^{b}	20.07 ± 3 ^{a}	19.03 ± 3 ^{abc}
3000	α-Tocopherol	0.0	47.13 ± 7 $^{g-j}$	45.40 ± 3 ^{j}	31.23 ± 3 ^{hi}	29.67 ± 2 ^{i}	21.07 ± 3 ^{e}	19.50 ± 3 ^{f}	16.17 ± 4 ^{hij}	14.30 ± 4 ^{l}
		25	47.13 ± 5 $^{g-j}$	45.53 ± 4 ^{ij}	31.33 ± 4 ^{ghi}	33.13 ± 4 $^{d-h}$	21.13 ± 1 ^{e}	19.57 ± 3 ^{f}	16.30 ± 4 ^{ghi}	14.23 ± 3 ^{l}
		50	49.70 ± 5 ^{cd}	47.90 ± 4 ^{efg}	33.23 ± 4 $^{d-h}$	31.67 ± 6 $^{e-i}$	22.60 ± 2 ^{d}	20.90 ± 3 ^{e}	17.33 ± 4 ^{efg}	15.17 ± 3 ^{jkl}
	Nicotinamide	2.5	47.23 ± 6 ^{ghi}	45.80 ± 4 ^{hij}	31.40 ± 4 $^{f-i}$	29.77 ± 5 ^{i}	21.10 ± 2 ^{e}	19.60 ± 2 ^{f}	17.20 ± 4 ^{fgh}	15.07 ± 3 ^{kl}
		5.0	48.87 ± 5 $^{d-g}$	47.50 ± 4 ^{fgh}	34.13 ± 4 ^{cd}	32.73 ± 4 $^{d-h}$	22.70 ± 1 ^{cd}	21.10 ± 1 ^{e}	17.80 ± 4 ^{def}	15.57 ± 3 ^{ijk}
5000	α-Tocopherol	0.0	37.93 ± 6 ^{l}	33.43 ± 6 ^{n}	23.17 ± 3 ^{jk}	22.13 ± 1 ^{k}	15.63 ± 3 ^{hi}	14.40 ± 3 ^{i}	11.40 ± 4 ^{no}	10.43 ± 2 ^{o}
		25	38.07 ± 7 ^{l}	33.53 ± 5 ^{n}	23.10 ± 5 ^{jk}	22.17 ± 2 ^{k}	15.70 ± 6 ^{hi}	14.50 ± 3 ^{i}	11.47 ± 3 ^{no}	10.53 ± 4 ^{o}
		50	40.37 ± 7 ^{k}	35.67 ± 4 ^{m}	24.80 ± 5 ^{j}	23.70 ± 2 ^{jk}	16.90 ± 6 ^{gh}	15.60 ± 4 ^{hi}	12.37 ± 3 ^{mn}	11.37 ± 4 ^{no}
	Nicotinamide	2.5	38.23 ± # ^{l}	33.53 ± 3 ^{n}	23.20 ± 5 ^{jk}	22.17 ± 3 ^{k}	15.73 ± 5 ^{hi}	14.43 ± 1 ^{i}	12.27 ± 3 ^{mn}	11.27 ± 4 ^{no}
		5.0	39.97 ± # ^{k}	35.20 ± 2 ^{m}	25.37 ± 5 ^{j}	24.00 ± 3 ^{jk}	17.13 ± 5 ^{gh}	15.73 ± 1 ^{hi}	12.77 ± 3 ^{mn}	11.67 ± 4 ^{n}

Free Amino Acids And Total Protein:

Amino acids acts as a putative osmoprotective solute leading to lowering osmotic potential in several tissues exposed to stress. The exposure of the two sunflower cultivars to salt stress induced an accumulation of free amino acids Fig. 2B. These results are in agreement with those observed by Azooz, (2004), Khattab, (2007), and Sadak *et al*. (2010). These results can be attributed to the decrease in protein synthesis and/or to the increase in its degradation. Furthermore, α-tocopherol or nicotinamide enhanced the stimulatory role of salt stress on production of free amino acids in sunflower cultivars. These results added support to the results obtained by Bassouny *et al.* (2008). Thus, it can be suggested that salt tolerance was manifested via activated proline synthesis Fig. 2C and hydrolysis of protein into free amino acids, which act as osmoprotectants in the two tested cultivars of sunflower plant. It could be concluded that the inhibitory effect of salt stress on the sunflower cultivars was alleviated by treatment of vitamins through enhancing the biosynthesis of free amino acids and their incorporation into protein.

Enzyme Activities:

The resistance to environmental stress may depend at least partially on the inhibition of ROS production and/or the enhancement of antioxidant level as well as the osmotic adjustment during seed germination and plant development. Salinity accumulates the ROS especially H_2O_2 in plant cells. The metabolism of H_2O_2 is dependent on various functionally interrelated antioxidant enzymes such as catalase and peroxidases. These enzymes are involved in elimination of H_2O_2 from stressed cells (Kim *et al*., 2005). Data presented in Fig. 3 showed that salinity stress caused reduction in the activities of CAT, POX, PPO and PAL enzymes. The reduction in catalase and peroxidases activities indicated that these enzymes were unable to completely neutralize H_2O_2 resulted from the oxidative salt stress (Shalata & Neumann, 2001).

Application of vitamins α-tocopherol and nicotinamide as seed soaking under the various levels of soil salinity caused marked increases in CAT, PPO, POX and PAL activities in sunflower plants as compared with the values of reference controls. Therefore, treatment with vitamin E and vitamin PP alleviated the adverse effect of salinity metabolic activities through decreasing the build-up of active oxygen species and thereby increasing resistance to salt stress (Hassenein *et al*., 2009). Also, α-tocopherol or nicotinamide can alleviate the

adverse effect of salinity via exhibiting high antioxidant activity of catalase and preventing the toxic accumulation of HO^-.

Regarding to PAL enzyme, there is a positive relationship between PAL activity and total phenolic compounds in sunflower plants. The hypothesis indicated that PAL activity was increased under salt stress and this enzyme is involved in the biosynthesis of phenolic compounds. Partially, the present results showed that soaking sunflower seeds in α-tocopherol or nicotinamide could improve the tolerance ability against salinity stress. Similar results obtained by Mohamed & Aly (2008).

Mineral Contents:

Salinity stress caused a considerable increase in sodium content, paralleled to decrease in nitrogen, phosphorous, calcium, magnesium and potassium contents of two sunflower cultivars Tables 2 and 3, under all salinity levels. In this connection, Zaho *et al*. (2007) and Kiarostami *et al*. (2010) suggested that increased accumulation of sodium (Na^+) and (Cl^-) ions in the tissues inhibits biochemical processes related to photosynthesis through direct toxicity and led to low water potential. The promotion of Na^+ uptake by salinity was accompanied by a corresponding decline in K^+ concentration, showing an apparent antagonism between K^+ and Na^+ (Cuin *et al*., 2009). The reduction in Ca^{2+} and Mg^{2+} uptake under salt stress conditions might be due to the suppressive effect of Na^+ and K^+ on these cations or due to reduced transport of Ca^{2+} and Mg^{2+} ions. In addition, salinity has an antagonistic effect on the uptake of Ca and Mg which caused by displacing Ca in membranes of root cells (Asik *et al*., 2009) on wheat.

High K^+/Na^+ ratio in Hysun 336 cultivar of sunflower plants than Euroflor cultivar may be explain the mechanism which caused to maintain high K^+/Na^+ ratio, either retention of K^+ or preventing Na^+ from accumulating in tissues is the key feature for salt tolerance in terms of biomass and grain yield (Chen *et al*., 2007). In this connection, Rahnama *et al*. (2011) reported that, Salt –tolerance genotypes sequestered high amounts of Na^+ concentration and maintained high K^+/Na^+ ratio. This ion partitioning may be contributing to the improved salt tolerance of genotypes.

In saline soil, Table 4 the solubility of micronutrient (Cu, Mn, Fe, Zn and Mo) is particularly low and plants grown in these soils often show deficiencies of this element (Page *et al*., 1990). High NaCl might affect iron absorption and might aggravate Fe deficiency or Fe toxicity (Yousef *et al*., 2007). Application of NaCl decreased Cu concentration of wheat plants, Turan *et al*. (2007). NaCl caused to decrease nitrogen, potassium, calcium and iron in the shoot tissue Turan *et al*. (2010).

Seed soaking in either Vit. PP or Vit. E. under the various levels of salinity caused a reduction of Na^+ accumulation and increase in the contents of K^+, Ca^{2+} and Mg^{2+}, as compared with the corresponding salinity levels. (El- Bassiouny, 2005 and El-Bassiouny *et al*., 2005). Vitamins led to increase in the contents of ions in the main organs of the stressed sunflower plants through their role in increasing osmotolerance and/or through regulating various processes including absorption of nutrients from soil solution. (Buschmann and Lichtenthaler, 1979). The antagonistic relations between Na^+ and K^+ may be taken as an indication of the role played by Vit PP or Vit E in modifying K^+/Na^+ selectivity under salt stress (Azooz, 2004).

Conclusions:

On the basis of present findings, it was concluded that there was no significant differences between two studied cultivars (Hysun 336 and Euroflor) in their response to sowing in saline soils at all physiological and biochemical parameters except at K^+/Na^+ ratio that explain increasing tolerance of Hysun 336 cultivar as compared with Euroflor cultivar. α-tocopherol (Vit E) or nicotinamide (Vit. PP) not only neutralized the effect of salinity stress but resulted in a significant improvement physiological and biochemical parameters as well as total contents of soluble sugars, phenols, proline, amino acids, and protein. Furthermore, soaking seeds in either Vit E or Vit. PP induced significant changes in the activities of both non-enzymatic and enzymatic antioxidants in sunflower plants grown at different salinity soils.

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